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The role of the microbiota in sedentary life style disorders and ageing: Lessons from the animal kingdom.

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Abstract

A paradox of so-called developed countries is that, as the major historical causes of human mortality are eliminated or mitigated by medical progress, life-style related diseases have become major killers. Furthermore, as life-span is extended by the combined effects of modern medicine, health-span is struggling to keep apace because of the burden of non-communicable diseases linked to diet and sedentary life-style. The gut microbiome is now recognized as a plastic environmental risk factor for many of these diseases, the microbiome being defined as the complex community of co-evolved commensal microbes that breaks down components of a complex diet, modulates innate immunity, and produces signalling molecules and metabolites that can impact on diverse regulatory systems in mammals. Aspects of the so-called "Western" life-style linked to disease risk such as energy dense diet and antibiotic treatment are known to affect the composition and function of the microbiome. Here we review the detailed mechanisms whereby the gut microbiome may modulate risk of diseases linked to sedentary life-style, and ageing related health loss. We focus on the comparative value of natural animal models such as

hibernation for studying metabolic regulation, and the challenge of extrapolating from animal models to processes that occur in human ageing.

Introduction to the mammalian microbiome

The term "microbiome" refers to the collection of microbes and their genes that is present as a community in a given location [1]. Some authors used the term "microbiota" to describe the microbes themselves, and "microbiome" to refer to their genes or collective coding capacity. The last decade has witnessed a dramatic surge in the application of microbiome studies across a wide range of fields, from environmental science, through to medicine and industrial microbiology. This is because modern tools in molecular biology and high-throughput sequencing have revolutionized the depth and precision with which complex microbial communities can be forensically studied [2]. Many of these tools were developed for environmental microbiology, where it has always been appreciated that complex communities of microbes that are hard to culture in the laboratory are often present. Human and animal body sites provide complex growth substrates to nutritionally fastidious microbes, which makes it hard to replicate these growth conditions in the laboratory, and many of these microbes were thought to be unculturable on laboratory media, but this has been disproved in recent years [3, 4]. Nevertheless, a major driving factor in the development and dissemination of microbiome technology was the ability to routinely and affordably catalogue the composition of the microbiome in a given location, and to study its correlation with states or traits. There are a diversity of ways to study the microbiome (summarized in Figure 1) that afford insights into what the bacterial community might do (its total coding capacity), the range of metabolites it produces (the metabolome), and the total complement of proteins detectable in a given sample (the metaproteome).

The pioneering studies in mammalian microbiome science were performed on humans, and a major focus was on the gut microbiome, which harbours one of the largest bacterial loads, and one of the most diverse bacterial communities [5]. In fact, the gut microbiome contains bacteria, fungi (mycobiome), eukaryotic viruses (virome), prokaryotic viruses (phageome), and protozoa, with most studies to date focussing on the bacterial component. The ELDERMET [8] established catalogues of microbes and their genes in various body locations. Curated databases

exist in which large numbers of standardized microbiome datasets from cases and controls are archived and which may be downloaded, facilitating comparative analysis [9].

Establishing what microbes are present in a biological sample can be performed quickly and cheaply by sequencing ribosomal RNA (rRNA) gene amplicons, whereas determining what functions these microbes encode requires true metagenomics, meaning shotgun sequencing of total community DNA [10]. Many of the early differences between microbiome datasets were due to technical variation in sampling, DNA extraction and preparation for DNA sequencing, a challenge in standardization that has been resolved through international collaborative effort to establish robust protocols [11].

The microbiome in human health and disease

The history of the discipline of microbiology was driven to a large degree by the need to understand microbes as pathogens, an endeavour that has encompassed some of the biggest milestones in the development of the scientific method [12]. The concept that microorganisms may be beneficial is also over a century old [13] but received relatively little attention until the late 20th century, when the beneficial effect of deliberately consumed single strains (probiotics) gained scientific and commercial traction [14]. A broad range of beneficial effects have been reported for probiotics but only a few mechanisms have been proven in humans, and even fewer claims granted regulatory approval [15]. The technical developments described above, that made identifying microbiome composition and function routine and affordable, facilitated a multitude of studies comparing the gut microbiome in various diseases and syndromes with that of healthy controls [16]. The core logic that these studies pivot upon is that the gut microbiome of an animal has co-evolved with that host species; that the microbiome provides a number of key functions including metabolism, innate immunity and colonization resistance; that deviations from harbouring the normal core microbiome to harbouring an altered microbiome (sometimes called dysbiosis [17]) associate robustly with particular diseases because of the loss of the relevant microbes [18]. The bidirectional interactions of the gut microbiome with external life-style factors which impact on it, as well as host physiological systems that the microbiome itself impacts upon, are summarized graphically in Figure 2. These effects are not all intestinal or metabolic; the gut microbiome has also been implicated in modulating behaviour and cognitive function, through its

effect on circulating neuroactive metabolites that bacteria either produce or deplete [19]. It logically follows on from these microbiome-disease associations that all patients suffering from the same microbiome-related disease should show a shared disease signature in their microbiome; that the altered functional properties of the microbiome should explain the mechanism of disease causation; that the disease may be rectifiable by providing the missing elements of the altered microbiome, or by repressing over-abundant microbes. The functions commonly associated with the gut microbiota, and the disease indications that arise when the microbiome is perturbed, have recently been reviewed [20]. Studies of microbiome disease associations are complicated by confounding factors that impact the microbiome (Figure 2) such as habitual diet [21, 22], antibiotics [23], medications [24], genetics [25] and geographical region [26]. It also appears that many diseases involving microbiome alteration share similarly altered microbiome signatures, and that only a small proportion of changes in the microbiome are actually disease specific [27]. Despite these challenges, microbiome research has underpinned a new wave of commercial endeavour aimed at developing taxa whose abundance is altered in particular conditions or diseases as Live Biotherapeutics [28].

Comparative analyses of human and animal microbiomes

Germ-free animals display a number of physiological, behavioural, immunological and metabolic impairments [29, 30], which support the notion that mammals and their microbiomes co-evolved in a symbiotic or mutualistic fashion [18]. Comparison of the composition and function of animal microbiomes could therefore elucidate the bacterial taxa and their functions involved in critical microbiome functions in particular mammalian hosts. These investigations confirmed that the relatedness of gut microbial communities in animals mirrored the phylogeny of the host organism but that diet patterns were very important, with the microbiome diversity of herbivores being much greater than that of omnivores or carnivores [31]. The bacterial relatedness networks were very concordant whether built upon the microbial taxa defined by ribosomal RNA genes or genome-wide coding capacity, showing that the metabolic functions also co-evolved with the animal host [32]. This prompted a re-appraisal of animal models for studying human gut microbiome-related traits, and the limitations of rodent models that display very different microbiome at phylum level to humans [30]. Short-chain fatty acids are produced by the

microbiome and are central to microbiome-host interactions, being used as an energy source for enterocytes, modulating host epithelial barrier function, proliferation and metabolism [33]. The major differences in the composition of the gut microbiome of humans compared to mice, rats and non-human primates are also reflected in the relative abundance of the major short-chain fatty acid levels in these hosts [34], underscoring the caution required when using animal models for studying primate traits. In large-scale comparison, both diet and body size affect microbiome diversity in the gut but gut physiology (fermenter versus simple stomach physiology) is the most important driver [35]. As a further caveat to be borne in mind when exploiting microbiome-mammal host co-evolution, a recent study demonstrated a discordance between gut microbiome composition and evolutionary history in primates, whereby the microbiome of humans consuming non-industrialized diets and monkeys subsisting on eclectic, omnivorous diets were more similar that other human-primate comparisons [36].

The ageing process

In recent times, ageing has been increasingly viewed as a collection of morbidities in the final decades of life that can be treated like a disease. Ageing, however, is more appropriately described as a process during which an accumulation of deficits takes place over the entire life-course [37]. It comprises a segmental and progressive loss of physiological function and physical capability over time, manifesting as a loss of physiological resilience and resultant frailty. It represents a dynamic interaction between an individual's genome, epigenome and exposome (i.e. social and psychological factors, nutrition, lifestyle and physical environment). Intuitively, this results in substantial interindividual variation in how we age and makes defining normative ageing difficult [38]. At a metabolic level it results in a general impairment of anabolic pathways and activation of catabolic pathways [39].

Features of ageing have been hallmarked to exemplify commonality across taxa [40], and accelerated biological ageing (i.e.'miles on the clock') is a feature of age-related morbidities that comprise a 'diseasome of ageing' [41]. In particular, dysregulated nutrient sensing is a hallmark of ageing, where epigenetic regulation of nutrient sensing pathways and nutritional differences tied to socioeconomic factors, show differential effects of the exposome on features of human ageing, such as age related genomic hypomethylation and inflammatory status [42, 43]. This diseasome

shares common features, including persistent low-grade inflammation, hyperphosphataemia, depressed Nrf2 activity, depleted metabolic capability, depressed mitochondrial biogenesis and alterations in the gut microbiome [39, 41]. These interactions are graphically summarized in Figure 3.

Of note, mammalian longevity shows a strong negative correlation with nutritionally acquired serum phosphate levels [44, 45]. Indeed, human progerias, such as Hutchinson's Guilford's Syndrome, or a disease of accelerated ageing, such as CKD, display elevated serum phosphate levels [44]. Epidemiological studies have indicated that frequent consumption of red meat is a source of age-related hyperphosphataemia in man [46]. This offers a mechanistic basis for age related health, inflammatory burden (inflammageing) and alterations in the composition and role of the microbiota. Red meat also contains carnitine, a substrate for production of the highly pro-atherogenic trimethylamine N-oxide (TMAO) from trimethylamine (TMA). Gut microbial enzymes are required for TMA production. TMAO, produced from TMA by liver enzymes, has been implicated in both ageing and mortality across a spectrum of age related disease [47-49] and may link read meat to mortality [50]. By extension, this is also pertinent to the relationship between inflammation and cancer. A pro-inflammatory phenotype is a feature of advanced biological ageing and associated with a range of cancers, in particular colorectal cancer [51]. Typically, this has been attributed to the production of a pro-inflammatory senescence associated secretory phenotype (SASP). Tumourigenesis, however, may be driven by an inflammatory microenvironment, even in the absence of overt inflammation. There is also a further emerging role for the microbiome in modulating both local and systemic inflammatory burden. Significantly, while the gut microbiome can contribute directly to the generation of pro inflammatory metabolites such as TMA, dysbiosis can lead to a reduction of butyrate production BORREL. Genomics and metagenomics of trimethylamine-utilizing Archaea in the human gut microbiome. *The ISME journal* 11: 2059). A reduction in such short chain fatty acid production can exacerbate inflammation and contribute to cancer progression by epigenetic dysregulation through microbial production of butyrate [52]. Notably, inclusion of more plant based protein into the diet has been reported to reduce systemic inflammatory burden and the incidence of both cancer and CVD related deaths [53]. This is supported by studies from the UK Biobank indicating an elevated risk of colorectal cancer associated with red and processed meat consumption [54]. It remains to be

determined how TMAO interplays with the exposome in normative ageing, though it is intuitive to predict that it will show dynamic interaction with the age-related microbiome.

While a link between food and health and longevity has been described from the time of the Celts and the mythical healing cauldron of *An Dagda*, through to the *Corpus Hippocraticum* of the Classical world, it regained attention in the early 20th century with studies on dietary restriction. The relationship appears both bi-directional and complex. Dietary restriction, for example, has repeatedly been demonstrated to improve health span and life span in pre-clinical models [55]. Critically, genetic heterogeneity appears to play a determinant factor in the effectiveness of such interventions [56], though the mechanistic basis is not well determined. The impact of epigenetic heterogeneity is still subject to intensive investigation [57].

Nutrition may also impact on the epigenetic landscape of ageing via the availability and provision of methyl donors for maintenance of the methylome [58]. This is consistent with reports indicating an imbalanced diet and low intake of fruit and vegetables correlates with genomic hypomethylation and accelerated biological ageing [59]. Additionally, recent observations have associated this with alterations in the microbiome (Craven and Shiels, in prep.). The impact of nutritional composition and metabolic sequalae influenced by the gut microbiome is an underpinning feature of metabolism across taxa and may even determine the relationship between energy storage and body mass [60].

Lack of dietary fruit and vegetable intake also provides a direct link to the production of alkyl catechols from dietary derived phenolic acids. Alkyl catechols production requires gut microbes and as such links microbial activity to cellular stress defences [61]. Significantly, alkyl catechols are potent Nrf2 agonists. Nrf2 regulates over 390 stress defence genes linked to longevity and health span. Moreover, alkyl catechols comprise a group of chemicals that include the senolytic agents fisetin and quercetin, which have proven efficacy in increasing lifespan and health span in mice [62].

The microbiome and ageing in *Homo sapiens* and in animals

Humans are born devoid of microbes, and neonates undergo a process of colonization in the early months of life that is marked by successive rounds of succession and extinction [63], finally resulting in a complex adult-like microbiome between 6 and 24 months of age, depending on

whether or not the infant was breast-fed, and at what age they were weaned [64, 65]. The healthy adult gut microbiota is individual-specific at strain level and stable over time, insofar as an individual subject's microbiome remains more similar to itself over time than it does to other subjects [66]. However, many studies have independently reported that the microbiome undergoes changes as a function of age [67-70], and we have shown that this age-related microbiome change is accelerated when it accompanies frailty, especially when promoted by consumption of a restricted diet [71-73]. Notably, a study of >1,000 subjects in China revealed very little difference of the microbiome in older subjects, perhaps because they were exceptionally healthy, lived in a single region, and ate a very similar diet [74]. Although lower levels of global microbiome diversity (alpha diversity) are common of older people, there is no threshold age at which this decline begins, and moving from a healthy high-diversity microbiome state to a frail low-diversity state may pass through an intermediate state of even higher diversity [71]. However, when subjects' microbiome were profiled longitudinally, temporal instability was more pronounced in subjects with low initial microbiota diversity [71]. Changes in the functional capacity of older subjects include loss of short chain fatty acid production associated with frailty [75, 76], increased tryptophan metabolism associated with reduced cognitive function in some centenarians [70], and higher levels of some methanogenic archaea that may protect against atherosclerosis in some older people [77]. The gut microbiome has been mooted as a target for improving health in older people [78]. However, until it has been definitely proven that restoring the gut microbiota of older people improves health or slows health loss, it is prudent to treat it for now as an environmental modifier of phenotype in a multifactorial disease process.

Relatively few studies have examined the microbiome of older animals, probably because laboratory animals are expensive to house, and in older life they often suffer high rates of disease associated with in-breeding and loss of heterozygosity. Studies in our laboratory of rats up to two years of age showed clear age-related microbiome alteration and increase in alpha diversity [79], with an increase in short-chain fatty acid production that contrasted with the decline in frail older human subjects [69]. Older mice also have a different gut microbiome to older mice, and interestingly, co-housing or fecal transfer from adult to aged mice alters microbiome and boosts germinal centre response [80]. The altered gut epithelial gene expression pattern of older mice [81] was correlated with differential abundance of particular species, and increased proportional

abundance of so-called pathobionts, species such as *Escherichia coli* and other Proteobacteria that are opportunistic pathogens. Impaired gut barrier function in older mice (evidenced in the previous study by gene expression analysis) was corroborated in another group by leakage of fluorescent markers from the gut lumen into the blood, and this correlated with impaired cognitive function and an altered gut microbiome [82]. Collectively these studies show the potential usefulness of comparative physiology and microbiome science to develop hypotheses and plan mechanistic investigations of microbiome-health links in ageing humans.

The gut microbiome and sedentary life-style disorders in *Homo sapiens*

The link between an altered gut microbiome and obesity was one of the earliest and most dramatic microbiome-disease links postulated [83]; unfortunately it is also one of the most complex [84, 85]. The first concept of how gut microbes might affect obesity centred on energy harvest from the diet by a microbiota enriched for phylum Firmicutes [86], which seemed be corroborated by microbiome alterations associated with weight loss during caloric restriction [87]. However other studies failed to replicate such simplistic microbiome remodelling with obesity [88], and there are now at least four microbiome-related mechanisms that probably contribute to obesity including calorie harvesting/energy metabolism, gut barrier function and inflammatory effects on adipogenesis, satiety and insulin resistance, and altered bile acid signalling [85]. This makes the task of finding a unifying microbiota signature for obesity very challenging [89], coupled with small sample size and lack of power that was a feature of many early studies [90].

Some obese subjects suffer also from impaired glucose metabolism [91] or metabolic syndrome, a spectrum of metabolic defects that respond to some degree to faecal microbiota transplantation [92]. Attempts to identify microbiota alterations for type 2 diabetes (T2DM) have been less consistent that obesity [93-95], due in part to the confounding effects of metformin that is very commonly used to treat T2DM [24].

Animal models have been useful in exploring microbial taxa and mechanisms involved in obesity and metabolic disorders. The ob/ob leptin deficient mouse was used to demonstrate the transmissibility of the obesity phenotype within germ-free mice [83], and in many follow up investigations. Various rodent (primarily mouse) transfer experiments [96] have also been performed to recapitulate metabolic disease phenotypes, and to test various interventions. The

variable success of human microbiota engraftment in wild-type or antibiotic treated mice, and the inflammatory response of germ-free mice to faecal transfer [97], complicate the interpretation of these experiments.

Animal models for microbiome and the diseasome of ageing

Human evolution has led to a basic physiology adapted to seasonal variation in both food type and availability, coupled with the development of an upward stance, bipedal gait and range mobility. Epigenetic regulation of metabolism has facilitated dynamic responses to changes in nutrient availability under such circumstances. Modern humans, however, have rapidly developed a capacity for sedentary living within an energy dense environment. A consequence of this is an increasing prevalence of obesity and associated metabolic sequelae, including T2DM, hypertension, fatty liver disease and chronic kidney disease and a range of cardiometabolic disorders [39, 98, 99]. Additionally, the human diet is atypical for the evolutionary development of the gut. Man is an omnivore, with dentition and a gut adapted from frugivorous primate ancestors for digestion of a wide range of plant-based protein. However, the modern Western diet, is excessively energy-rich and has a disproportionately large intake of red meat [100]. This skewing towards a more carnivorous diet has resulted in a dietary association with the diseasome of ageing. Excess red meat consumption has been linked with hyperphosphataemia, renal dysfunction and cancer [101, 102], while reduced fruit and vegetable intake has been associated with microbiome changes, poorer physiological resilience and adverse cardiovascular health [103].

Pre-clinical modelling of the impact of the microbiome on human health is fraught with the danger of artefact and error [104]. This stems from both inadequate mammalian models for human health and differences in microbial diversity between species. However, murine models while typically best fits for conditions of human health, share not only the hallmarks of ageing, but also common mammalian elements of ageing, such as hyperphosphataemia and inflammageing. Despite differing life strategies, murine models have proven invaluable in dissecting metabolic disorders and ageing. Additionally, they have the advantage of being able to be produced in germ free conditions, to enable more subtle understanding of the impact of the microbiome on human health and unlike worms and dipterans, share both anaerobic and aerobic commensal organisms.

Significant manipulation of the microbiome in models of accelerated ageing has yet to be undertaken, though one obvious choice for such an investigation is the klotho mouse [105]. The klotho gene encodes a transmembrane protein that acts as an obligatory co-receptor for FGF23, a regulator of phosphate metabolism. Mutation in klotho results in progeria and an associated range of features common to the diseasome of ageing, including renal dysfunction, neurodegeneration, vascular dysfunction, arteriosclerosis, sarcopaenia, osteoporosis [106]. What such models do not address with respect to human health is the impact of a sedentary lifestyle and inter-individual variation in response to differences in nutrition [56].

While providing rodents with calorie excess can cause obesity and generate pathogenic sequelae, they do not recreate the aetiopathogenesis of human sedentary associated disease. The human exposome, for example, and its influence on disease predisposition and progression, is not captured by standard laboratory models. The exposome will undoubtedly have species specific effects engendered on different species living in the same physical environment. The level of psychosocial stress within that environment has a direct impact on both ageing and adiposity in man [43] and would thus be expected to have a similar impact on any model organism and to be reflected in differential epigenomic effects (e.g. methylation patterns). Notably exposome changes radically affect gut microbiota [107].

A range of epidemiological studies have indicated that dietary risk factors in humans have distinct genetics associated with them and that the gut microbiome differs in response to the same diet in obsess and lean individuals. Translating this from a rodent model, where intra-strain differences have substantial metabolic differences is challenging to say the least [108]. One possible solution is to investigate common genera/OTUs associated with underlying ageing processes and how these interplay with the epigenome of ageing in response to dietary variation. By this means one can then link environmental differences (i.e. nutrition) to age related changes in health.

Nonetheless, microbial transplantation studies in rodent models have generated exciting data that modulation of microbiota may improve management of Type I diabetes (T1DM) [109]. Notwithstanding the caveats outlined above, rodent studies have implicated *Bacteroides* spp. as a major associated factor in T1DM-associated microbial dysbiosis.

Microbial transplantation from obese humans to lean mice has been demonstrated to confer obesity on recipient animals [110]. This has also been observed in xenotransplantation of pre-hibernation bear microbiota to germ free mice, resulting in the recipients becoming obese and torpid [111]. Non-human primates are more closely related both physiologically and genetically to humans than other model organisms, but their use is complicated by a range of concerns including cost, the time it takes to do meaningful studies and relatively low subject numbers. They are, however, a critical translational bridge going from small animal preclinical models to human clinical trials. Non-human primates are prone to the development of obesity and a range of associated comorbidities, but in the wild this is rare due to their levels of physical activity and a diet that is rarely in energy excess. When fed a high fat, and, or a high sugar diet, they develop adiposity, diabetes and a range of adverse cardiometabolic features, including hypertension [49, 112, 113]. Strikingly, captive primates develop a 'humanized microbiome [107] possibly linked with a shift in diversity or types of dietary polysaccharides.

As such, the change in exposome in going from the wild to captivity, or being reared in captivity, bears a striking parallel to the increasing prevalence of cardio-metabolic disorders in modern man and the effect of mass human migration and Western diet on the composition of the human microbiome, As such, non-human primate model may represent a 'gold standard for assessing the impact of the microbiome on human health span. It is notable, that with the humanisation of captive primate microbiomes, comes a correlation with the appearance of cardiovascular disease and psychopathologies not typically observed in their wild counterparts [114, 115].

One aspect of human health not typically covered by traditional preclinical models is the impact of hospital stays in Intensive treatment units, where the patient is bed bound and often subject to *ex vivo* support for organ function. The consequence of such prolong intensive support is loss of bone and muscle mass and eventual organ failure. These features are not observed in hibernating mammals, such as the brown bear [44], which is able to preserve muscle and bone mass during several months of hibernation. Critical to this is a dynamic microbiome [111]. The composition of the bear microbiome alters seasonally as the bears prepare for hibernation, paralleling seasonal nutritional diversity. This has consequential metabolic sequalae, such as fat

accumulation. Germfree mice colonized with bear microbiota adapted the seasonal physiological characteristics of the bear, clearly indicating a role of the microbiome in physiological obesity.

No mention of models of ageing can go without discussion of the naked mole-rat (NMR). The NMR has a lifespan exceeding 30 years and appears to defy Gompertzian laws, as their mortality rate does not increase with age [116]. They are resistant to neoplasia and oxidant stress, display minimal changes in age-related physiological function or tissue deterioration and remain reproductively active throughout their lifespan. The composition of their gut microbiome appears to reflect their subterranean existence and is rich in bacteria able to use soil sulphate as a terminal electron acceptor for anaerobic oxidative metabolism [117]. Notably, their microbiome shares features with human centenarians and Hadza hunter-gatherers, which have been proposed as models for a human gut microbiome relative to normative ageing processes, including a high load and diversity of *Spirochetaceae* and the presence of *Mogibacteriaceae*. Significantly, NMRs exhibit an enhanced capacity to produce short chain fatty acids associated with reduced colonic inflammation. Additionally, they have an enhanced capacity for production of mono- and disaccharides, which may aid in fructolysis in an oxygen deprived environment and enable resistance to oxygen deprivation.

Knowledge gaps and key future experiments

While animal models undoubtedly provide critical insight into disease pathogenesis and offer application for experimentation impossible for conventional human studies [118], they typically involve laboratory rodents that are metabolically morbid and lacking appropriate features of either human pathogenesis or normative ageing [119, 120]. Such models are sedentary, obese and glucose intolerant, which while appropriate for the study of human obesity, or aspects of metabolic syndrome, such as T2DM, they still exhibit a poor correlation with the human disease [110]. This is also observed for hypertension, which is now the most common disease globally [120]. Recently, however, a mini pig model has been described which more closely described the human pathogenesis of metabolic syndrome, inclusive of adipose tissue inflammation and adipocyte necrosis, not see in other rodent models [121]. This is encouraging, but still has a fundamental flaw, in that captivity will alter its co-evolved commensal microbiome, as has been observed for primates. Little is also known as to whether there is a seasonally adjusted

microbiome in response to available food stuffs in the wild. As such, the best model for the study of humans remains human subjects.

However, looking more widely in the animal kingdom, hibernating species and microbiome dynamics associated with significant metabolic reprogramming necessary to maintain health during periods of hibernation, offer excellent models to study the impact of nutrition on the microbiome and how this is linked to physiological function. This is exemplified by the Brown bear and the Giant Panda. Notably, gut microbial transition also occurs in Pandas translocated from captivity to wild habitat and has a direct bearing on the survival of this species. Understanding any relationship between the symbiotic gut microbiome and host environmental adaptation in this context, is not only important for the species survival and conservation, but offers a novel model system for insights into the underpinning molecular mechanisms in a normative (or near normative) environment [122]. How this will translate to human exposome differences related to health span remains to be determined.

Conflict of interest statement

PWOT is a founder of 4D Pharma Cork Ltd., a company developing microbiome diagnostics and live biotherapeutics. As a PI in APC Microbiome Ireland, he has pursued collaborative research with General Mills (cited in ref. 97). Neither of these relationships have materially influenced the content of this review.

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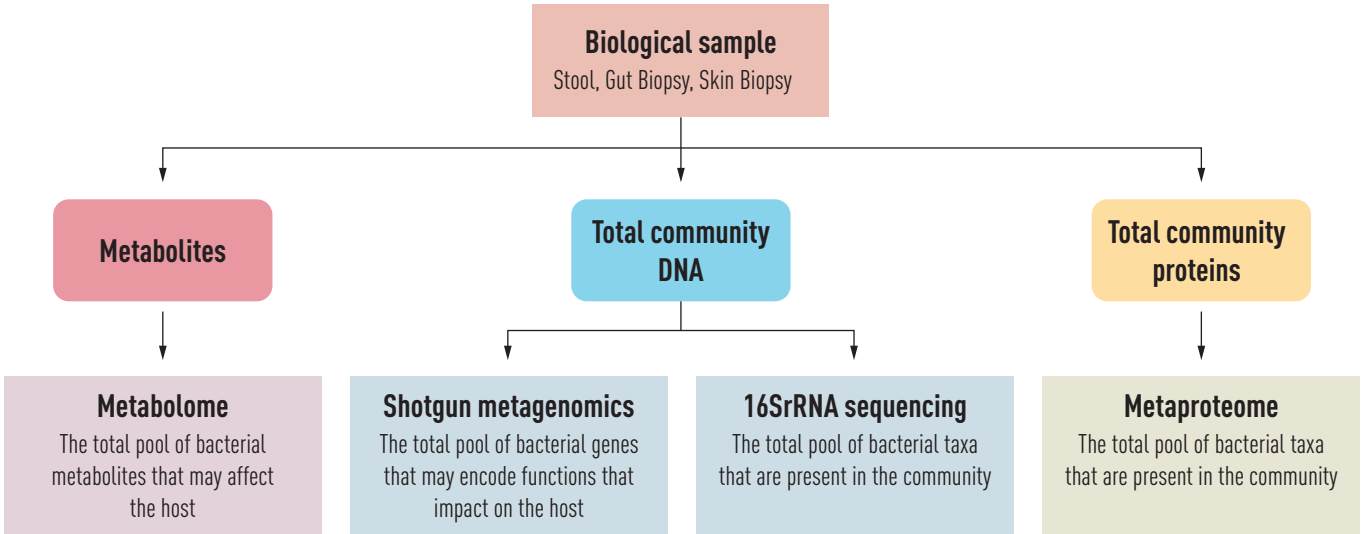
Figure legends

Figure 1. Methods for studying the microbiome and associated read-outs.
Exposome factors modulate the micorboiome in older sedentary humans, in turn directly modualring factors regualting health span

Figure 2. Schematic representation of the bidirectional interaction of the human gut microbiome with external factors that it is modulated by, and host systems that it may influence. The challenge is to identify natural animal systems that emulate these interactions, or model systems where they can be manipulated to explore mechanisms

Figure 3. Interaction between environmental modifiers, the microbiome, and health
The exposome represents the geophysical and social environments of an individual across their lifecourse. This has a direct impact on an individual's microbiome, particularly via the composition and calibre of the foodome. A sub-optimal foodome can lead to an aberrant microbiome with consequential health effects, typically mediated by accelerated ageing, whereby the hallmarks of normative ageing become dysregulated, metabolic morbidity is facilitated and the diseasome of ageing is ensues at an earlier chronological age.

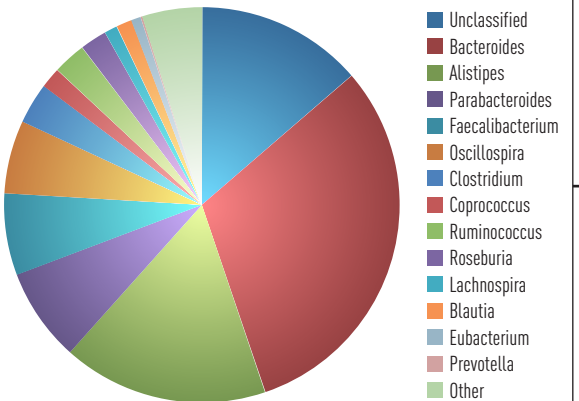
Methods for studying the microbiome and associated read-outs



Microbiome modulators in the exposome

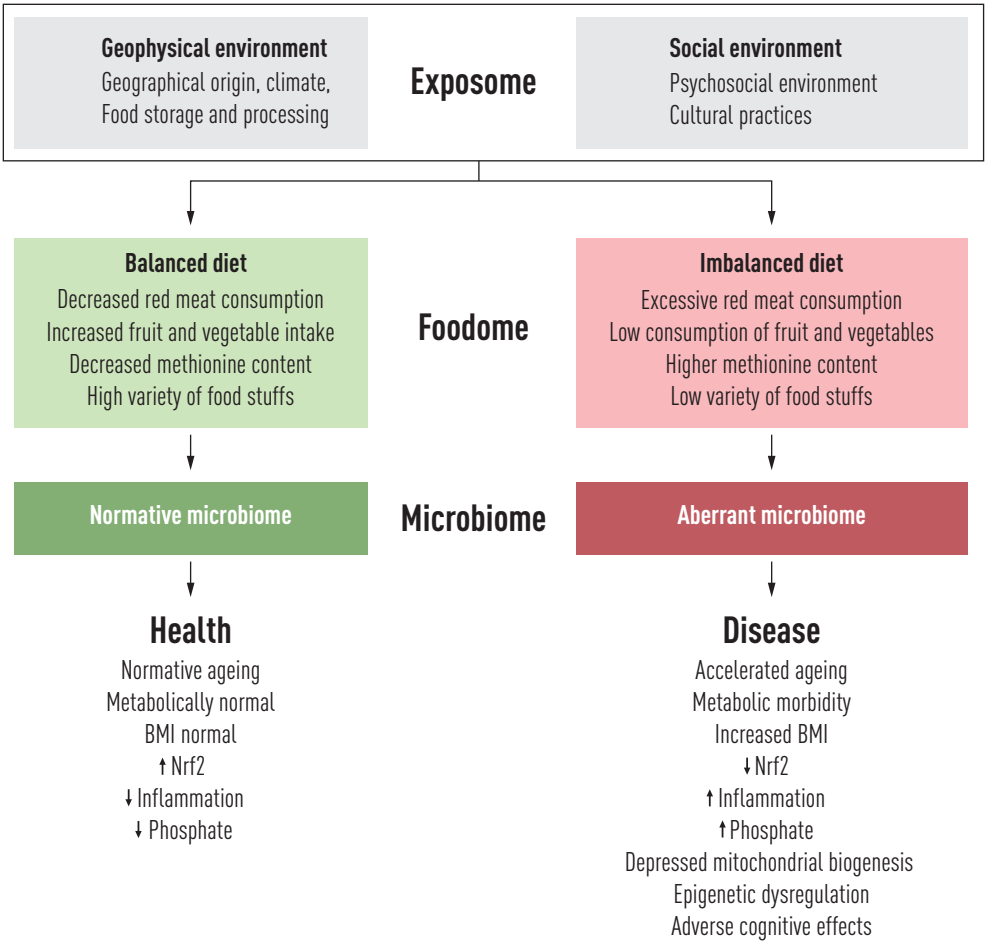
Geography
Diet
Medication: Antibiotics, Metformin, Statins, Proton pump inhibitors, Other medications
Psychosocial factors
Life-style, exercise
Surgery/radiation

Gut Microbiome of Sedentary Older Human



Host effects

Colonization resistance (pathogens)
Nutritional e.g. SCFA production, vitamins
Signalling e.g. bile acid regulation, adipogenesis, satiety, epigenetic effects
Innate immune modulation e.g. inflammation
Behavioural e.g. neuroactive metabolites


Geophysical environment
Geographical origin, climate,
Food storage and processing
Exposome
Social environment
Psychosocial environment
Cultural practices
Balanced diet
Decreased red meat consumption
Increased fruit and vegetable intake
Decreased methionine content
High variety of food stuffs
Foodome
Imbalanced diet
Excessive red meat consumption
Low consumption of fruit and vegetables
Higher methionine content
Low variety of food stuffs
Normative microbiome
Microbiome
Aberrant microbiome
Health
Normative ageing
Metabolically normal
BMI normal
↑ Nrf2
↓ Inflammation
↓ Phosphate
Disease
Accelerated ageing
Metabolic morbidity
Increased BMI
↓ Nrf2
↑ Inflammation
↑ Phosphate
Depressed mitochondrial biogenesis
Epigenetic dysregulation
Adverse cognitive effects